# NONSPECIFIC ENHANCEMENT OF HOST FACTORS IN RESISTANCE TO STAPHYLOCOCCAL DISEASE*

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RESISTANCE to staphylococcal disease is the resultant of an equation involving both the staphylococcus and man. Stephen L. Morse has discussed some of the staphylococcal contributions to this equation. Let us now consider the contributions of the host. Table I, which is modified from one by Janeway,[1] lists some of the more important factors that are involved.

Under the heading of "barriers" we include those at the surface and in the tissues. Man is normally protected by an intact skin with an acid *p*H, an adequately functioning mucous blanket over the respiratory and gastrointestinal membranes, a good flow of tears with sufficient lysozyme, a surface microbial population which is benign, tissue factors which limit the growth and survival of staphylococci, and facial surfaces which may trap them.

The ability of phagocytes to capture and destroy staphylococci is dependent first upon an adequate inflammatory response to penetration of surface barriers. This permits an early release of polymorphonuclear leukocytes into the tissue spaces for phagocytosis. The destruction of the engulfed staphylococci is in turn dependent upon the ability of the granules of the phagocyte to release functioning enzymes into vacuoles that contain the bacteria. This process is enhanced by the presence of complement.

Lymphoid cells—both lymphocytes and plasma cells—must be present and capable of recognizing the staphylococci in order to produce hypersensitivity and antibodies. For a long time immunologists have had techniques for quantitating staphylococcal antibodies. Consequently

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TABLE I.—HOST FACTORS IN RESISTANCE TO STAPHYLOCOCCI

1. Barriers—on surface and in tissue
2. Phagocytes—for capture and destruction of bacteria
3. Lymphoid cells—to recognize antigens and produce hypersensitivity and antibodies

these have been studied much more extensively than the cellular factors. This is unfortunate since cellular factors now seem to play a highly important role in man's defense against staphylococcal disease.

Having listed some of the host factors which operate in the staphylococcal resistance equation, we may ask *whether they can be influenced by nonstaphylococcal means.* The answer, of course, is that they are. We know that resistance is lowered in favor of the staphylococcus when the surface barriers are broken. This is seen with burned or injured skin, when mucosal cells are injured by viruses or irritating gases, or when tear ducts are occluded. Staphylococcal infections are also more frequent in individuals with neutropenia, splenic deficiency, or congenital dysphagocytosis. We know that patients with agammaglobulinemia do not handle staphylococci well. All these are examples in which the host's defenses are depressed. We now must ask the more important question: *can these resistance factors be raised by nonspecific means?* I need not remind this audience that it is not necessary to kill off all the staphylococci in order to prevent disease. We need only reduce their number below the critical minimal concentration required to establish a lesion. Thus it would seem reasonable that if we could maintain an intact and healthy skin and mucous membrane, we might prevent penetration by large numbers of staphylococci. Good skin care and nutrition are therefore obvious nonspecific means of helping the host to resist staphylococci. "Bacterial interference" as utilized by Marvin Boris and his co-workers serves also to raise a surface barrier to invasion by pathogenic staphylococci. The patient with marked neutropenia or agranulocytosis is often helped by putting him on reverse isolation to reduce his exposure. The patient with IGG deficiency handles staphylococci better if he is given monthly injections of gammaglobulin. Unfortunately, no practical method has yet been found for increasing—even for short periods—the capture-and-destroy activity of man's phagocytes. However, there are a large number of substances

TABLE II.—ENHANCED RESISTANCE IN ADULT MICE TO INTRACEREBRAL CHALLENGE WITH STAPHYLOCOCCI

| *Pretreatment* | |
|---|---|
| *Saline* | *SE* |
| 48 survivors | 110 survivors |
| 198 total | 199 total |
| Survival 24% | Survival 56% (difference of 32%) p = <0.001 |

with which this can be done in animals. These include endotoxins in proper dosage, BCG and cellular components of mycobacteria, a variety of large molecular polysaccharides, and DNA fragments. Most of these substances have multiple biological activities which preclude their use in man. Nevertheless, the fact that phagocytic competence can be increased in animals suggests that some day, with other materials, it may be possible in man.

For several years, both at The Mount Sinai Hospital and at North Shore Hospital, our group has been studying the resistance-enhancing activity of a substance which we prepared from the supernatant of a trypticase soy-broth culture of the Bartlett strain of staphylococcus (SE).[2,3,4] It appears to be a polysaccharide or mucopolysaccharide containing a ribitol, a hexosamine, and a nitrogen group. Although it is obtained from a staphylococcus, its effectiveness is not limited to staphylococcal infections. When it is given intra-abdominally it protects suckling and adult mice and rabbits against subsequent lethal challenge, by a variety of routes, against several strains of staphylococcus. It also protects mice against *Escherichia coli* and herpes simplex virus. SE is not effective *in vitro*. It requires time to work. It must be given at least four hours before challenge and the protection which it elicits persists for only about 48 hours. However because it is nontoxic, it has been a useful tool in our study of phenomena associated with nonspecific resistance to infection.[5]

On this occasion I should like to present the results of some experiments with this substance in the adult mouse. They will serve only to illustrate the importance of cellular responses in these mice when they are made more resistant to staphylococcal infection.

When the SE substance is given intra-abdominally it results in the

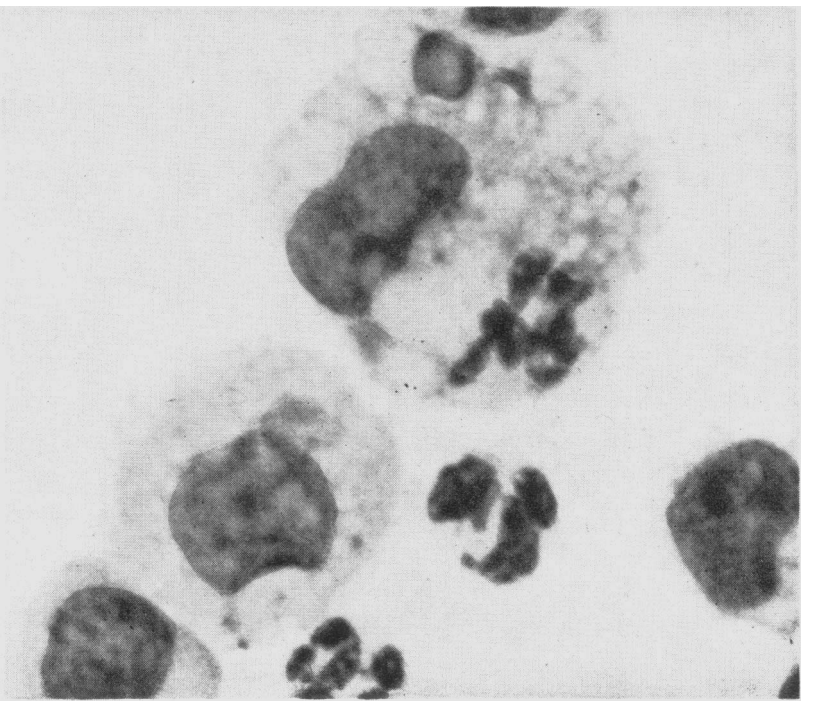

Fig. 1. Mouse peritoneal cells 48 hours after intra-abdominal injection of 6 mg. of SE. The increase in size and vacuolation of cytoplasm in the mononuclears is striking.

protection of a significant number of mice against intracerebral challenge with staphylococci (Table II). How this protection actually is achieved is not entirely clear. However, we do know that SE elicits a series of obvious quantitative and qualitative changes in the peritoneal leukocytes.[6] The qualitative changes are most interesting. A smear of peritoneal cells from an unstimulated mouse shows mononuclear cells predominantly. Intra-abdominal injection of pyrogen-free saline does not change this picture. However a similar smear made *three hours* after the intra-abdominal injection of 6 mg. of SE shows that an increased number of cells and polymorphonuclears predominate. *Twenty-four hours* later the polys are less prominent and about 30 per cent of all the cells are large vacuolated mononuclears. After *48 hours* the number of polys continues to decrease and the mononuclears show an increase in size and degree of vacuolation (Figure 1). After 72 hours the peritoneal smear is like that of the unstimulated mouse.

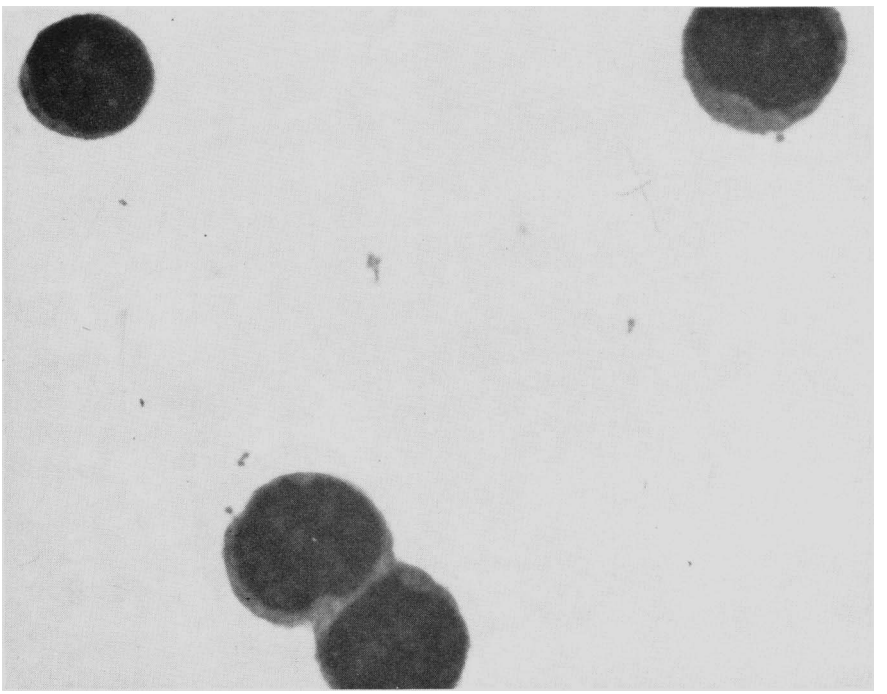

Fig. 2. Peritoneal smear from control mouse 15 minutes after the intra-abdominal injection of staphylococci.

These changes suggest that the metabolic activity of the cells have been stimulated somehow by the injected material. The changes in the mononuclears resemble those described by others after injection of endotoxins.[7]

When mice are stimulated by SE intra-abdominally and then challenged by staphylococci intra-abdominally there is more prompt phagocytosis and digestion of the bacteria by the already mobilized leukocytes.

Figure 2 is a representative smear of peritoneal cells from a control mouse 15 minutes after the intra-abdominal injection of a large number of staphylococci. There is no phagocytosis. It was not until after two hours that active phagocytosis was seen in these animals and after 24 hours there were still some cocci visible within the polymorphonuclears.

Figure 3 is a smear from a mouse pretreated 20 hours earlier with SE, also taken 15 minutes after intra-abdominal challenge. The phago-

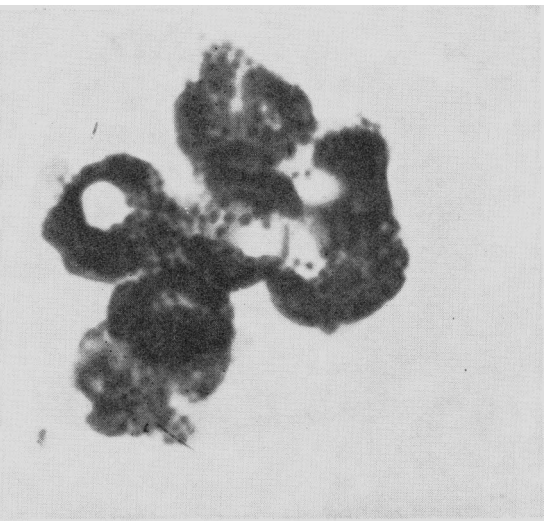

Fig. 3. Peritoneal smear from a mouse pretreated intra-abdominally with SE taken 15 minutes after the intra-abdominal injection of staphylococci.

cytosis is striking. After 24 hours no cocci were seen either free or within the polymorphonuclears.

When mice stimulated intra-abdominally with SE are challenged by staphylococci at a distant site, intracerebrally, there is a more rapid accumulation of leukocytes at the site of challenge than in the controls. This is best shown by impression smears of the brain made after two hours.[6]

These observations suggest to us that the intra-abdominal administration of the resistance-enhancing substance SE has a systemic as well as a local effect. The changes which were seen in our experimental model may not have been the only cause for increased survival in the treated mice, but they do show that it is possible to enhance the activity of mouse leukocytes artificially and nonspecifically.

What we need is something comparable for man. If this were available and even if its effect were of short duration, it might be given at the time of high risk—e.g., immediately after birth or before surgical operation, and might be a significant factor in raising the resistance of the patient.

I should like to end with a reference to Derrick Rowley, who has worked long in this field. In a recent paper[8] he emphasized that the

first few hours of contact between the host and its bacterial parasite were decisive—even though the interaction might continue for several days. He estimated that an increase of from 95 per cent to 99 per cent in the "competence" of phagocytic cells would be sufficient to reverse a picture unfavorable to the host to one in his favor.

What we need is a safe and effective stimulus to increase the competence of phagocytes. Perhaps we are really asking for a new kind of nonspecific chemotherapy—one directed at leukocytes rather than at bacteria.

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